

**WORK DESCRIPTION**  
**Department of Animal and Aquaculture Sciences, NMBU**

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**Method name: Kjeldahl-N**  
BIOVIT No. : Arb1040

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### 1. Introduction

The Kjeldahl method is used to determine the quantitative amount of nitrogen ( $\text{NH}_4^x$ ) in a sample. It was originally developed in 1883 by the Danish chemist Johan Kjeldahl to determine the protein content of grains.

The method is an internationally recognized reference method for determining the protein content of feed. It can be used for almost all types of samples such as food, raw materials, water, sludge, concentrates, roughage, fish feed, grain and fertilizers. The method involves decomposing the amino acids in the protein using high temperature, strong acid (concentrated sulfuric acid) and a catalyst (copper sulphate).

It is important to be aware that the Kjeldahl method is unable to measure the total nitrogen content of organic samples. Nitrogen atoms in oxidized nitrogen ( $\text{NO}_x$ ) and nitrogen atoms in heterocyclic compounds (cyclic organic compounds containing at least one atom other than carbon in the ring chain) are not determined.

### 2. Reagents

- Cold losses (3.5 g  $\text{K}_2\text{SO}_4$  + 0.4 g  $\text{CuSO}_4$ ).
- Concentrated sulfuric acid,  $\text{H}_2\text{SO}_4$  (> 95%)
- Boric acid with methyl red and bromocresol green
- Sodium hydroxide, NaOH (35%)
- HCl (0.2 M)
- RO water

### 3. Risk assessment

Sulfuric acid is supplied using a manual pump that is constantly on the jug. The piston is pulled up (set to 15 mL), slowly pressed down while holding the hose against the glass wall of the test tube (to supply the concentrated sulfuric acid extra carefully). After adding sulfuric acid, the hose must be higher than the liquid level in the sulfuric acid container so that sulfuric acid does not drip into the fume cupboard.

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Lift the drip tray on the cooking block **BEFORE** the samples are put on. This **MUST** be done so that the sulfuric acid gas is extracted from the flue.

**NB!** Wear safety goggles, thick acid gloves and a lab coat! **NB!**

Sodium hydroxide and hydrochloric acid are corrosive. In case of spillage on clothes, these must be removed immediately and the skin rinsed with large amounts of water. In case of spillage on bare skin, rinse with large amounts of water. The boric acid solution is very dilute and has no danger label.

#### 4. Equipment

- Analysis weight (0.0001g)
- Cooking block (Kjeltec 2520 Auto Digester, Tecator)
- Kjeltec Auto TM 8400
- Kjeldahl blocks w / Kjeldahl pipes.
- Weighing boats

#### 5. Sample material

It weighs in approx. 0.3 - 1.0 g sample depending on the protein content (see table). Dry samples are weighed in weighing vessels and transferred to Kjeldahl pipes, while raw samples are weighed directly in the pipes.

Weight test (g)	Content of protein (%)
0,3	>50
0,5	25-50
1,0	3-25

Blank samples go through the same procedure as the samples.

#### 6. Special remarks

#### 7. Job description

1. Screw on block (program Protein 3001). Before turning on the Scrubber, change the water in the water flask. (Tap). Check the color of the lye mixture, should be sky blue. Turn on the Scrubber.
2. Turn on Kjeltec. (It runs self-test about 3 min.)  
Check reagents (lye, boric acid, hydrochloric acid, water.)

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3. Dry samples are weighed in weighing vessels and transferred to Kjeldahl pipes. Crude samples are weighed at weight C, directly in Kjeldahl tubes.
4. Check that the scale has the correct program for weighing in Kjeldahl-N.  
 Press application, system, peripherals.  
 Printer must be set to OFF, Host must be set to RS232, OK exit.  
 Enter launch Compass on PC, notification appears, press YES.  
 Login: Username: Admin  
 Pin code: 1234 OK.  
 Touch the new batch icon (top left). Enter block number on batch name. It should say kjeldahl on Analysis type. Press 20rack 250 ml on Rack name.  
 Click on samples at the top. Tube 1 is always blank ml, press OK for the number of blanks we want. If we are going to give 3 blanks, press OK three times and try the last blank (tube 4). Press Show details, sample type: convert blank to sample, result type: convert ml to mg N / g.  
 Enter ID on the test: ID ex: Requisition number 36-1 etc.  
 Place the pointer in the field next to Get, when the weight is stable press Get. Check that the weight has entered. The program automatically goes to the next pipe, enter the next ID.  
 If it is not a full block, remember to press “delete sample” on the last sample that appears. Press Save, press Send batches.
5. Add 2 pcs. Kjeltabs for the samples, including the blank samples. Boiler losses should not be added to the empty pipes. Carefully add 15 mL of concentrated H<sub>2</sub>SO<sub>4</sub> to the tubes.
6. When the block is 420 degrees, hang the block on the lift, remove the drip tray, press Start. Boil for 1 hour, cool the tubes for 15 min. over the hob before removing it. Cool the samples for 20 min.
7. Put on the drip tray on Kjeltec Digester and unscrew the scrubber and cooking block.
8. The samples are now ready for analysis of nitrogen at Kjeltec TM 8400.

### Analysis on Kjeltec TM 8400

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9. Press Assign, press Wing out, insert the block (check that it fits snugly, press it down firmly).

If there are several blocks on the display, press the block to be run.

Press Assign at the bottom right.

Press Analysis on the display, press Start (red arrow at the bottom right).

When the block is finished. Go to PC and transfer results.

Press: get all files for selected instrument,

Press: OK.

### 7. Reporting and calculation of the analysis result

Result is stated as mg N / g sample, but the results are sent out to the client as g N / kg sample.

$$mg \frac{N}{g} test = g \frac{Nkg}{kg} test$$

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